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ETHYLENE - AN ENDOGENIC SUBSTANCE IN TUMOR VECTORS

[Following is the translation of an article by M. T. Kokonov of the Biochemical Laboratory of the State Research Institute of Oncology imeni P. A. Gertsen, Moscow, in Voprosy Meditsinskoy Khimii (Problems in Medicinal Chemistry), Vol. VI, No. 2, March-April 1960, pages 158-165.]

Scientific facts accumulated on carcinogenesis give a basis for the assumption that with the formation of tumors due to natural causes in a number of cases there appear chemical substances of an endegenic origin. The nature of these substances is not known.

An exploration carried out by the author earlier of ponds in which a spontaneous mass appearance of cancer in fish gills was discovered showed that in the gas emanating from the bottom slime and dissolved in the pond water the ethylene content reached 3 to 4 percent (usually in ponds the ethylene content is only 0.3 to 0.5 percent). Experiments carried out under laboratory conditions on the influence of ethylene and a few of its primary derivatives (ethylene oxide) on fish (Gambusia) under the particular conditions caused cancer of the fish gills. A connection between mass sickness of fish in ponds due to malignant tumors and a high content of organic residues is pointed out in literature[1].

It has been shown[2] that under the action of ethylene in plants a tumor-like growth of undifferentiated tissues is formed. Microorganism Phitomonas tumefaciens which in plants produces "crown

gall" tumors and gives off gaseous ethylene has been isolated from tumors in man and mice[3, 4]. It has been shown by many authors[5 - 10] that the primary derivatives of ethylene (ethylene oxide, ethylene glycol and others) possess strong mutagenic and carcinogenic properties.

Other authors have discovered[11 - 13] that aqueous solutions of ethylene react easily with albumin and β-globulin in mild conditions whereby 1 mole of egg-albumin or β-lactoglobulin can combine with 80 - 120 moles of ethylene oxide. At the same time the reaction is shifted 1 - 3 pH units to the basic side. Under these conditions the resulting protein - ethylene oxide compounds are insoluble dissociate with difficulty when the reaction medium is made acidic or basic. These reactions are irreversible. It has been established that ethylene oxide interacts extremely vigorously with carboxyl- and sulfhydryl - groups.

It has also been shown[14 - 16] that ethylene oxide interacts with such products of living organisms as ammonia and amines forming ethanolamine and, particularly choline. Upon decomposition of choline trimethylamine, ethylene glycol, and ethylene oxide are formed. In the work by Michel[17] it was experimentally shown that upon decomposition of choline in the animal intestine trimethylamine is formed. Theoretically also ethylene glycol and ethylene oxide should be obtained. 'Dent and Walshe[18] found ethanolamine in urine of the animal afflicted with the primary stage of cancer of the liver and for a period of 7 months observed its separation from the urine.

There is basis to assume that ethanolamine in such a large quantity in the organism of the tumor-carrier was formed from endogenic ammonia or ethylene oxide, but not from serine upon its decomposition.

A liver afflicted with a huge tumor (the weight of the tumor was 13.1 kg. and comprised about 1/4 of the total weight of the sick animal), probably, lost its ability to transform ammonia into urea and it could accumulate in great quantities. From ammonia and ethylene oxide ethanolamine must have been formed. Normally in urine, as is known, no ethanolamine is detected.

On the basis of the facts above it can be assumed that the formation of tumor growth is possibly the result of an interaction of several primary derivatives of ethylene (ethylene oxide, ethylene glycol and others) with the proteins of the living organism, in the result of which the synthesis of the protein substance is distorted. Probably, these ethylene derivatives form endogenously in the organism under the influence of several external as well as internal factors from such precursors as choline and ethylene; from the first one by decomposition, from the other one by acidification. Ethylene in turn, probably, forms in the living organism endogenously in the same manner as this take place in the higher plants and fungi.

In this report data are given on an experimental investigation of the problem of the possibility of endogenic formation of ethylene in the living organism under normal conditions and upon affecting the organism with malignant tumors.

Methods of Investigation

The work was performed with white male rats weighing about 100g. and partly with ascarids. The following groups of rats were used: healthy; healthy, exposed to ultraviolet light; healthy, pretreated with small doses of ethylene; healthy, after a subcutaneous injection of aluminum oxide; sick, with a subcutaneous inoculation of sarcomas; sick, with subcutaneous abscess. The animals were kept in

cages under ordinary diet. For inoculation of tumors the sarcoma strain M - 1 was used. Swine ascarids were used.

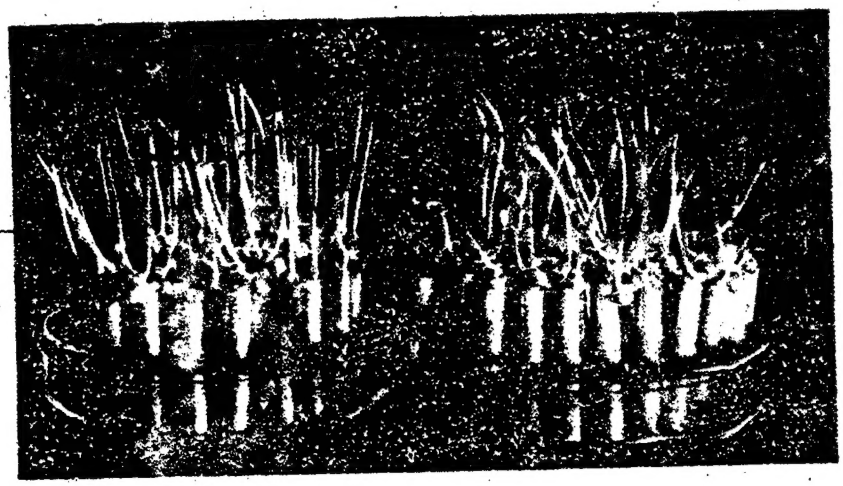

Fig. 1. Boxes with Vicia sativa sprouts.

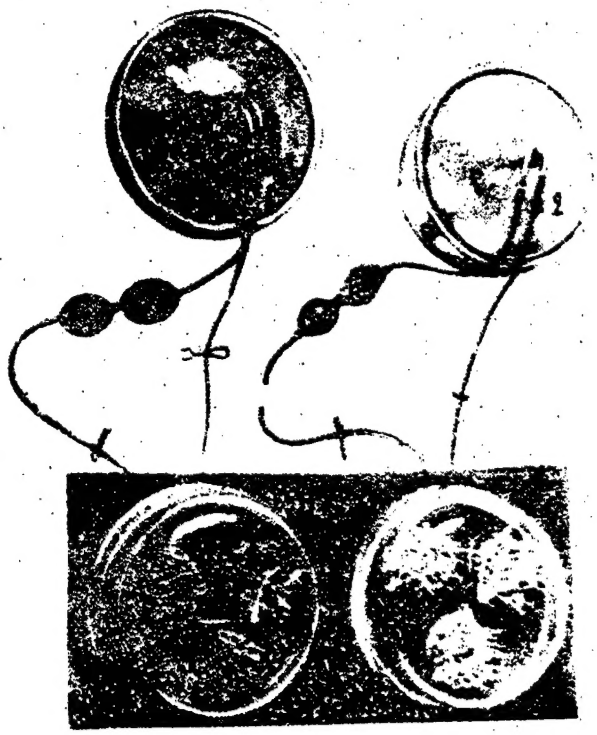

Fig. 2. General view of the instrument for ethylene determination given off by animals.

Basically the methods as developed and applied by the auther for determination of small concentrations of ethylene (applicable to animal objects) in air exhaled by animals were adapted from methods developed for detection of small doses of ethylene in plants.

As an indicator for ethylene were used ethylated sprouts a pure variety of the white-grained, vernalized Vicia sativa "h - 10" grown on distilled water in darkness at 20 - 22° from swollen seeds subjected to a vacuum under water to pressures to 0.5 - 0.3 cm of Hg residual pressure. Sprouts were picked according to the length of their primary roots (radix) within ± 1 mm. with the optimal length of 10 - 20 mm. and placed in 40 mm. long glass tubes; the tubes were mounted in boxes with 50 tubes in each. Tubes made of filter paper were placed in the glass tubes, they had the same length as the glass ones (Fig. 1). The boxes with the lower ends of the tubes down were immersed into Petri dishes containing a mineral salt solution suggested by D. N. Pryanishnikov: 1) NH_4NO_3 - 1.20 g.; 2) $MgSO_4$ - 0.3 g.; 3) KCl - 0.60 g.; 4) $CaSO_4$ - 0.72 g.; 5) $CaHPO_4$ - 0.66 g.; 6) $FeCl_3$ - 0.125 g. in 5 l. of aqueous solution. The test animals in groups of three were placed into 8 liter dessicators equipped with tubes. Using tubing passing through the tubes the dessicators were connected with similar dessicators in pair each pair forming a closed system. In the other dessicator the biological indicators were located(Fig. 2).

A CO_2 absorber was connected into the system between the animal dessicator and the dessicator containing the indicators. Through this system with the help of a Richardson bulb air from the animal vessel was pumped into the vessel containing the indicators and returned via other tubing for the total time of two hours (air was exchanged 25 times). After this the vessels containing the indicators were disconnected with the tubes having been closed beforehand and kept at

20 - 22°in darkness for 24 hours. After 24 hours the vessels containing the indicators were aerated for 15 minutes again hermetically connected into the system and the procedure with animals repeated; after this the vessels were allowed to stand for 48 hours. Altogether the experiment lasted 70 - 72 hours. After a specified time the indicators were removed from the vessels and the length of the epicotile and the radix of the growths were determined by a ruler.

In each variant of the experiments 200 - 500 indicators were used. Altogether 95 experiments with 3 variants per experiment were carried out. The figures obtained for the growth of the sprouts after appropriate statistical processing were compared with each other. Inhibition of the growth of the sprouts in the experiment, when compared with a control, indicated the presence of ethylene; the degree of inhibition, when compared with standard inhibition due to the action of ethylene, showed, however, the concentration of ethylene in the atmosphere of the instrument vessels. The reliability of the experiments was calculated according to the following formula:

$$\frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}$$

, where M - the arithmetical mean for the length of the growth, but m - the mean error of the arithmetical mean.

In order to convince ourselves that by using a biological indicator we actually did detect ethylene, air was passed through a chamber with 10 - 15 rats inoculated with sarcoma M - 1 five to fifteen days earlier; the air was subsequently passed through a cooled (1 - 5°) Millon's reagent for 57, 30, and 27 days. Subsequent treatment of the reagent with hydrochloric acid evolved a gas which was collected in ampules. Analysis of the collected gas samples was carried out using a mass-spectrometer by prof. N. N. Tunitskiy and the senior research assistant

M. V. Tikhomirov in the adsorption process laboratory of the L. Ya. Karpov Physical-Chemical Research Institute.

Table 1

Inhibition of the growth of Vicia sativa "h - 10" sprouts in relation to the concentration of ethylene gas in the atmosphere.

Experiments No of test	①	Ethylene concentration	Reading of	Growth in mm. (average) Test	Control	②	$\frac{M_1-M_2}{\sqrt{m_1^2+m_2^2}}$
82—II	4	1:600	E[1] R[1]	4±1,0 5±1,5	62±4,0 48±4,0	93,5 89,6	14,5 10,1
82—III	4	1:3000	E R	4±1,0 7±1,3	62±4,0 48±4,0	93,5 85,5	14,5 10,0
82—IV	4	1:6000	E R	4±0,8 8±1,5	62±4,0 48±4,0	93,5 83,4	14,5 10,0
82—V	4	1:16000	E R	4±0,5 12±2,0	62±4,0 48±4,0	93,5 75,0	14,5 9,0
82—VI	4	1:24000	E R	4±0,5 15±2,5	62±4,0 48±4,0	93,5 68,7	14,5 7,3
83—II	4	1:100000	E R	6±1,5 19±3,5	90±3,0 57±2,5	93,4 66,7	24,0 9,5
83—III	4	1:500000	E R	22±1,0 51±3,0	90±3,0 57±2,5	75,6 12,3	22,7
83—IV	4	1:1000000	E R	32±2,0 51±3,0	90±3,0 57±2,5	64,5 12,3	16,6 1,5
83—V	4	1:5000000	E R	77±3,5 51±2,6	90±3,0 57±2,5	14,5 12,3	—
83—VI	4	1:10000000	E R	81±4,0 51±1,5	90±3,0 57±2,5	10,0 12,3	—
83—VII	4	1:50000000	E R	88±4,0 54±2,0	90±3,0 57±2,5	2,9 5,3	

[1] E — epicotile; R — radix.

1) Number of repetitions;
2) Inhibition of growth in test compared with control in percent.

Results of the investigation

In Table 1 typical data are given for characteristic magnitudes of the inhibition of growth of the Vicia sativa sprouts in relation to ethylene concentration in the atmosphere. According to these data the standard curve was constructed with which the experimental data were compared.

From tables 1 and 2 it is evident that more sensitive toward small ethylene concentrations, in the order of $1:10^6$, are the primary roots (radix) of the Vicia sativa "h - 10" sprouts as compared with the epicotile. In botanical studies of ethylene only the reaction toward the epicotile is used[20, 21, 23].

In our study we considered basically the reactions toward the radix, and in passing also the reaction toward epicotile.

From Table 2 it is evident that healthy rats (control II) form endogenously and exhale ethylene into the surrounding atmosphere in a quantity of about 17 - 20 microliters to 1 kg. of live weight in a 24 hour period during the first 15 days of the experiment; from the 15th to the 20th day (for 5 days) the exhalation of ethylene increased twofold. Tumorous rats within the first ten days after inoculation (test I) exhaled ethylene in a quantity exceeding 5.4 times the exhalation from healthy rats (contol II). Furthermore, with the appearance of necrosis and up to the time of the ulceration of the tumors (20th day after inoculation) the quantity of ethylene exhaled by sarcomatous rats gradually fell and on the 20th day(ulceration of tumors) arrived at the original quantity (control II, up to 15 days).

Tables 3 and 4 represent the results of tests of two other series. These data confirm the regularity of the increased ethylene production by tumorous animals and its decrease at the time of the ulceration of the tumors.

Production of ethylene by the healthy control animals from the 15th

Table 2

Comparative Dynamics of Ethylene Exahalation by Tumorous Rats (Sarcoma M - 1) in Relation to the Progress of the Sickness (Inhibition of the Growth of Vicia sativa "h - 10" Sprouts).

№ опыта (1)	Число повторностей (2)	Время роста опухолей в днях (3)	Учтено по (4)	Прирост проростков в мм (5)			Торможение роста проростков по отношению к контролю III (6)				Количество этилена, выделившегося за 24 часа на 1 кг живого веса крыс (в мкл) (7)	
				I опыт (8)	II Контроль (9)	III контроль (10)	в %		$\frac{M_1-M_2}{\sqrt{m_1^2+m_2^2}}$			
				Саркоматозные крысы (11)	здоровые крысы (12)	без крыс (13)	I	II	I	II	I	II
60	4	4	E	75±3	80±3,5	87±3	13,8	8,0	2,8	1,5		
			R	43±2	51±2,5	61±2	29,5	16,4	6,4	3,1	43	18
7 61	12	10 - 11	E	60±2,5	76±2	80±2	17,5	5,0	4,4	1,4		
			R	38±2	50±2	60±5	36,7	16,7	11,0	5,0	92	17
62	4	14	E	65±2,5	69±2	82±2,5	22,0	15,9	5,1	4,0		
			R	41±2	51±3	62±3	33,9	17,7	5,8	2,6	52	17
8 63	14	16—17	E	41±2,5	38±2,5	48±2,5	14,6	20,8	2,0	2,8		
			R	40±2,5	44±2	59±2,5	32,2	25,4	5,4	4,7	42	25
9	6	20	E	34±2	34±2	41±1,5	17,1	17,1	3,5	3,5		
			R	37±1,5	35±1,5	50±2,5	26,0	30,0	4,3	5,0	21	36

[1] E — epicotile; R — radix.

1) Test number; 2) number of repetitions; 3) time of tumor growth in days: 4) reading of.; 5) growth of sprouts in mm; 6) inhibition of growth of the sprouts in relation to control III; 7) the quantity of ethylene which separated in 24 hours per 1 kilogram of the life weight of rats (in mcl); 8) I test; 9) II control; 10) III control; 11) rats with sarcoma; 12) healthy rats; 13) no rats.

to the 20th day of the experiment increased twofold as compared with the preceeding days (Table 2). We assumed that the reasen for this was a catalytic action of the increased concentration of ethylene, exhaled by the sarcomatous rats, on the healthy rats located in a cage next to a cage with the sarcomatous ones. The basis for such an assumption were data from work of a number of research-botanists, which showed that ethylene possesses a clearly expressed autocatalytic property for its formation by plant tissues. For the explanation of this question we carried out another series of experiments with 7 time points for ethylene determination, data for which are given ih Fig. 3. Shown on the graph is the comparative dynamics of ethylene exhalation by rats with subcutaneous non-infectious abscesses.

Table 3

The comparative dynamics of ethylene production by rat's organism during the growth of inoculated sarcoma M - 1 (intense tumor growth).

Age of tumors in days	Tumor stage	Inhibition of growth of Vicia sativa sprouts in percent compared to control		Reliability	
		epicotile	radix	epicotile	radix
4	Growth	20	12	3.9	3.5
12	Necrosis	35	33	6.5	5.9
16	Necrosis, and	0	6	—	1.5
22	Ulceration	0	0	—	—

Table 4

The comparative dynamics of ethylene production by rat's organism during the growth of inoculated sarcoma M - 1 (intense tumor growth), (slow in tumor growth)

Age of tumors in days	Tumor stage	Inhibition of growth of Vicia sativa sprouts in percent compared to control		Reliability	
		epicotile	radix	epicotile	radix
10	Growth	9	30	2.2	5.6
15	Onset of necrosis	21	31	3.8	5.5
20	Necrosis	29	21	4.5	3.9
25	Necrosis, ulceration	27	28	5.6	6.5
30	Necrosis, ulceration	17	13	3.5	2.5

As can be seen, the healthy rats (control III) which were isolated from the experimental rats during the entire experimental period (20 days) uniformly exhaled ethylene in a quantity of 18 microliters per 1 kg. of live weight in a 24 hour period. However, those healthy rats, which were given ethylene by way of inhalation for 2 hours (once only) 5 days in advance of the experiment in a concentration of $1:10^4$ in air (control II), by the beginning of the experiment, i. e. 5 days after innalation, and to the end of the experiment exhaled 143 microliters of ethylene per 1 kg. of live weight in a 24 hour period, i.e. 8 times as much as the controls (III).

Healthy rats, weighing 80 - 85 g. and more, 24 in number, and exposed to ultraviolet light once a day (except holidays) for 20 minutes and for a period of 8 months, did not produce ethylene during the time when the control animals exhaled 18 - 20 microliters of ethylene per 1 kg. of weight in a 24 hour period.

Healthy animals, after having been given three subcutaneous injections of 1 ml. of a 20 percent aluminum hydroxide suspension each time, from the 5th day after the last injection exhaled 8 - 10 times more ethylene than the controls. The determinations were carried out once a week for 2 months, but thereafter every month for 10 months. After 11 -12 months

eight out of the fifteen experimental rats developed tumors - sarcomas - at the location of the subcutaneous injection. Several tumors reached a weight of 70 g., the total weight of the animal being 200 g. All the rats that developed tumors were affected lightly.

The quantities in Table 5 fit the assumption that live swine ascarids liberate in 24 hours per 1 kg. of weight 16 microliters of ethylene.

In Table 6 the result of the verification by mass-spectrometric analysis of the gas is shown, as determined by a biological indicator to be ethylene, after a continuous exhalation of it by sarcomatous rats (57, 30, and 27 days), as well as concentrations.

The results obtained indicate that the endogenic formation of ethylene is inherent in rats and ascarids. Considering the fact that a number of authors have shown the possibility of ethylene formation by many higher plants and fungi, it can be assumed that this phenomenon is inherentalso in many forms of animals, and in their number , also in man.

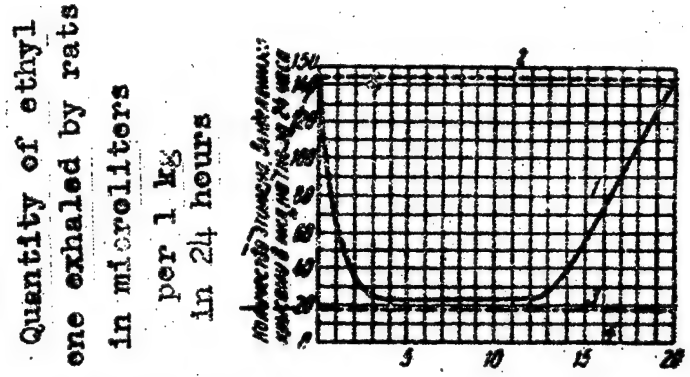

Fig. 3. Comparative dynamics of ethylene exhalation by rats with subcutaneous abscesses (by rats of variants I and II which were given ethylene 5 days in advance of the experiment)

1 - rats with subcutaneous abscesses (test 1)

2 - healthy rats (control II)

3 - healthy rats (control III)

4 - control (without rats)

The rats with the non-infectuous subcutaneous abscesses (test 1), for the whole period of sickness until the full healing of the wounds caused by the abscesses, completely lost the ability, caused by inhalation of ethylene by the animal, to produce an increased quantity of ethylene as compared with the control (II).

Table 5

Inhibition of Growth of Vicia Sativa Sprouts Due to the Emanation of Ethylene Liberated by Ascarius cuum

(1) № опыта	(2) Повторность	(3) Число индикаторов	(4) Прирост radix (в м.м)		Торможение роста radix в опыте по сравнению с контролем (5)		$\frac{M_1 \quad M_2}{\sqrt{m_1^2+m_2^2}}$
			(6) контроль	(7) опыт	(8) в м.м	(9) в %	
80	14	12	57±3	47±2	+10±3–2	18	2,9
80	14a	18	60±1	44±3	+16±3–1	27	5,3
80	14б	60	59±2	48±3	+11±3–2	19	3,1
(10) Среднее	3	90	59±2	46±3	+12,3	21	3,8

1) Test No.;
2) Repetitions;
3) Number of indicators;
4) Growth of radix in mm;
5) Inhibition of radix growth in the test as compared with the control:
6) Control;
7) Test;
8) In mm;
9) In percent;
10) Average.

Table 6. Results of a mass-spectrometric analysis of gases of the air circulated through a chamber containing tumorous rats.

Components	Contents in percent				
	Test			Control	
	No.1	No.3	No. 4	No.2	No. 5.
	after 57 days	after 30d.	aft. 27 d.	after 57 d.	aft.30d
Ethylene	79,6	79,5	75,4	0,88	0,0
Nitrogen	9,88	18,8	22,2	83,9	94,75
Other gases	0,52	1,7	2,4	16,22	5,25

Several external factors, probably, being catalysts can increase the endogenic formation of ethylene in the organism, but other factors of the oxidizing type, possibly transform ethylene into its oxide and other derivatives.

Ultraviolet light, X-rays, and radioactive radiation, being the strongest oxidizing factors, possibly, act on the organism as carcenogens by transforming ethylene into its oxide.

Conclusions

1. Healthy animals form ethylene endogenously and exhale it into the surrounding medium at a rate of 17 - 20 microliters per 1 kg. of weight in 24 hours.

2. After animals are subcutaneously injected with tumors the production of ethylene increases 4 - 5 fold, during the period of the beginning of the disintegration of the tumor the exhalation of ethylene decreases and by the time of ulceration drops to the original quantity (17 - 20 microliters)

3. Healthy rats after inhalation of ethylene acquire the ability to produce ethylene in increased quantities as compared with controls.

4. The formation of subcutaneous abscesses in animals takes away their ability to exhale ethylene into their surrounding medium.

5. Animals after receiving a subcutaneous injection of a suspension of aluminum hydroxide increase the production of tehylene as compared with controls.

6. Healthy animals after exposure to ultraviolet light decreased the amount of ethylene exhaled three times as compared with the control.

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